IMMUNO-DOT BLOT ASSAY

(Wright & Morton, 1989)

INTRODUCTION

This assay may be used as a positive or negative test for presence of glomalin but not for determining concentration. It also gives a permanent result, since the color is on the nitrocellulose membrane and does not fade.

MATERIALS

0.2 um nitrocellulose membrane
petri dish or dish to incubate the membrane in
2% non-fat milk (2g powdered milk/100ml PBS)
PBS (phosphate buffered saline)
PBST (PBS with Tween 20)
1% BSA (1 g BSA/100 mls PBS) (Make about 500-1000 mls of stock and dispense in 6 ml aliquots that will be frozen until needed.)
TBS (Tris buffered saline (20mM tris and 250mM NaCl)
MAb32B11 antibody (diluted with PBS, typically 1 ml in 5 mls PBS)
Biotinylated anti-mouse IgM (4.8 ul/6 ml 1% BSA)
ExtrAvidin peroxidase (3.0 ul/ 6 ml 1% BSA)
Color developer (make just prior to using)
    0.015g 4-chlor-1-naphthol in 5ml ice cold methanol,
    right before use add 25 ml TBS + 15ul of 30% hydrogen peroxide
dissecting needle
shaker or tilt table

METHODS

(1) Mark the membrane into small squares with the dissecting needle
(2) Place 1ul of undiluted sample in each square
(3) Block membrane with 2% non-fat milk by shaking for 15 min.
(4) Add diluted MAb32B11 and incubate on shaker for 1 hr.
(5) Remove antibody, add PBST and incubate on shaker for 5 min. Repeat PBST incubation twice.
(6) Incubate diluted biotinylated anti-mouse IgM with the membrane for 1 hr on shaker.
(7) Remove IgM, add PBST and incubate on shaker for 5 min. Repeat PBST incubation twice.
(8) Add diluted ExtrAvidin peroxidase and incubated for 1 hr on shaker.
(9) Remove peroxidase, add PBST and incubate on shaker for 5 min. Repeat PBST incubation twice and once with TBS.
(10) Develop with color developer until color is seen. Remove and dry. Store dry at room temp.